

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :		A1	(11) International Publication Number: WO 97/05169
C07K 14/74, 16/28, A61K 38/16, C12N 5/08			(43) International Publication Date: 13 February 1997 (13.02.97)
(21) International Application Number:	PCT/NL96/00183	(81) Designated States:	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date:	25 April 1996 (25.04.96)		
(30) Priority Data:	95202039.4 25 July 1995 (25.07.95)	EP	
	(34) Countries for which the regional or international application was filed:	AT et al.	
(71) Applicants (for all designated States except US): RIJKSUNIVERSITEIT TE LEIDEN [NL/NL]; Stationsweg 46, NL-2312 AV Leiden (NL). THE UNIVERSITY OF VIRGINIA PATENT FOUNDATION [US/US]; Towers Office Building, Suite 1-110, 1224 West Main Street, Charlottesville, VA 22903 (US).		Published	With international search report.
(72) Inventors; and			
(75) Inventors/Applicants (for US only): GOULMY, Els, A., J., M. [NL/NL]; Blijdorpstraat 2, NL-2343 BC Oegstgeest (NL). HUNT, Donald, F. [US/US]; 970 Old Ballard Road, Charlottesville, VA 22901 (US). ENGELHARD, Victor, H. [US/US]; 1401 Old Ballard Road, Charlottesville, VA 22901 (US).			
(74) Agent: SMULDERS, Th., A., H. J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).			

(54) Title: HA-2 ANTIGENIC PEPTIDE

(57) Abstract

The present invention discloses the first peptide sequence of a so-called minor H antigen. The minor H antigens are associated with the Graft versus Host Disease. The peptide and its derivatives find many uses in bone marrow transplantation, organ transplantation and in the treatment of leukemia. The peptide and its derivatives can be incorporated in vaccines, in pharmaceutical formulations and they can be used in diagnostic test kits. The peptide is derived from the HA-2 minor antigen and has the sequence YXGEVXVSV, wherein X represents a leucine or an isoleucine residue. Both donors and recipients in bone marrow transplantation can be treated with the peptides, optionally in combination with other peptides, coupled to carriers, with suitable excipients and/or adjuvants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Larvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

Title: HA-2 antigenic peptide

This invention relates to the field of immunology, especially cellular immunology. It particularly relates to the area of transplantation of organs, tissues or cells (especially bone marrow) and possible immunological reactions 5 caused by such transplantations.

Bone marrow transplantation (BMT) finds its clinical application in the treatment of, for instance, severe aplastic anaemia, leukaemia and immune deficiency disease.

In the early days many of these transplantations resulted 10 in rejection of the transplant or in Graft versus Host Disease (GvHD). It is nowadays clear that these effects are largely due to the presence of major H antigens which function as a major transplantation barrier. Consequently, improved success in bone marrow transplantation was reported when matching for 15 the HLA antigens was taken into account. Nowadays, mainly HLA-identical siblings or HLA-matched unrelated individuals are used as a source for the donor material. Still, despite the improvements in HLA-matching, as well as improvements in pretransplantation chemotherapy and/or radiotherapy and the 20 use of potent immunosuppressive drugs as prophylaxis, as well as better antibiotics and better isolation techniques for the donor material, about 20-70% (depending on their age) of the recipients still suffer from GvHD. In GvHD immunocompetent donor T cells react against the host tissues. Therefore 25 donation of marrow from which the mature T cells have been

removed has become a frequently used regimen. However, this often leads to graft rejection or failure, as well as to recurrence of the original disease, which is particularly dramatic in leukaemia.

5 The problems still associated with (particularly) human transplantation can hardly be attributed to the major H antigens, since the donor and recipient are routinely screened for HLA identity. Therefor GvHD may be caused by the disparity of the products of the so called 'minor' H systems (mHag),
10 i.e. Histocompatibility antigens other than those encoded by the MHC.

mHag's have been originally discovered in tumour- and skin rejection studies between congenic strains of mice. Over 40 mHag loci have been defined, dispersed over the genome, but
15 estimations predict the existence of several hundred loci. One of the better known minor H antigens is the HY antigen.

Several reports have demonstrated the presence of anti-host mHag specific CTL in patients suffering from GvHD after HLA genotypically identical BMT (1-7). In our laboratory, much
20 effort was put into the further characterization of a (small) number of anti-host mHag specific CTLs. Hereto, CTL clones specific for host mHag were isolated from the peripheral blood (PBL) of patients suffering from severe GvHD (8).

Subsequent immunogenetic analyses revealed that the CTL
25 clones (as described above) identified five non-sexlinked mHag, designated HA-1, -2, -3, -4, -5, which are recognized in classical MHC restricted fashion (8). mHag HA-3 is recognized in the presence of HLA-A1 and mHag HA-1, -2, -4 and -5 require

the presence of HLA-A2. Segregation studies demonstrated that each of mHag HA-1 to HA-5 is the product of a single gene segregating in a Mendelian fashion and that HA-1 and HA-2 are not coded within the HLA region (9). The mHag differ from each 5 other in phenotype frequencies; mHag HA-2 appeared very frequent (i.e. 95%) in the HLA-A2 positive healthy population (10).

With regard to the mHag expressed on different tissues, we observed ubiquitous versus restricted tissue distribution 10 of the mHag analysed (11). The expression of the mHag HA-2 is restricted to the cells of the haematopoietic cell lineage, such as thymocytes, peripheral blood lymphocytes, B cells and/or monocytes. Also the bone marrow derived professional antigen presenting cells; the dendritic cells and the 15 epidermal Langerhans cells express the mHag HA-2 (11, 12). The mHag HA-2 is also expressed on haematopoietic stem cells (13), on clonogenic leukemic precursor cells (14), as well as on freshly isolated myeloid and lymphoid leukemic cells (15).

To substantiate the importance of the human mH antigenic 20 systems, we investigated whether the mHag are conserved in evolution between human and non human primates. Therefor, cells from non human primates were transfected with the human HLA-A2.1 gene. Subsequent analyses with our human allo HLA-A2.1 and four mHag HLA-2.1 restricted CTL clones revealed 25 the presentation of ape and monkey allo and mHag HY, HA-1 and HA-2 peptides in the context of the transfected human HLA-A2.1 molecule by ape and monkey target cells. Furthermore, peptides were eluted from HLA-A2.1 molecules expressed on the

transfected ape cells. An HA-2 positive fraction was identified that showed the same behaviour on reverse phase HPLC as the HA-2 fraction derived from human EBV-LCL. This implicates that the HA-2 peptide is conserved for at least 5 35 million years (16).

A prospective study was performed in order to document the effect of mHag in HLA genotypically identical BMT on the occurrence of acute (grade ≥ 2) GvHD. The results of the mHag typing using the CTL clones specific for five well defined 10 mHag HA-1 to HA-5 demonstrated a significant correlation between mHag HA-1, -2, -4 and -5 mismatch and GvHD (17).

We aimed at the biochemical characterisation of human mH antigens. Thereto, we made use of the immunopurification and biochemical techniques successfully applied by Rammensee and 15 his colleagues (18, 19) to extract murine mH peptides from MHC molecules. Indeed, HPLC separation of low Mr molecules (< kD) obtained from acid treated MHC class 1 HLA-A2.1 molecules appeared successful. Fractions with sensitizing activity for 20 the non-sexlinked mh antigen HA-2 specific CTL clones were isolated (20). To analyse the peptidic nature of the mHag HA-2, two sets of experiments were carried out. First, the sensitizing activity of the mHag containing fractions, obtained as described above, is susceptible to protease treatment; i.e. incubation of these mHag containing HPLC 25 fractions with pronase or proteinase K abolished the sensitizing activity (21). Second, the MHC encoded TAP1 and TAP2 gene products are required for mHag peptide presentation on the cell surface. The transporter genes TAP1 and TAP2

associated with antigen presentation are required for delivery of peptides from the cytosol with the endoplasmic reticulum (22). The availability of a human celline "T2") lacking both transport and proteasome subunit genes enabled us to study the 5 processing and presentation of human mH antigens. We demonstrated that the (rat) transport gene products TAP1 and TAP2 were required for processing and presentation of antigenic peptides from influenza virus and from the intracellular mH protein HA-2 (23).

10 However, until the present invention no one has succeeded in identifying amino acid sequences of antigenic peptides relevant in the mHag system, nor has anyone succeeded in the identification of the proteins from which they are derived. We have now for the first time identified a peptide which is a 15 relevant part of mHag HA-2:

Thus this invention provides a (poly)peptide comprising a T-cell epitope obtainable from the minor Histocompatibility antigen HA-2 comprising the sequence YXGEVXVSV or a derivative thereof having similar immunological properties, wherein X 20 represents a leucine or an isoleucine residue.

The way these sequences are obtained is described in the experimental part. An important part of this novel method of arriving at said sequences is the purification and the choice of the starting material. Said novel method is therefor also 25 part of the scope of this invention. However, now that the sequence is known, it is of course no longer necessary to follow that method, because the peptides can easily be made synthetically, as is well known in the art. Since routine

techniques are available for producing synthetic peptides, it is also within the skill of the art to arrive at analogs or derivatives of the explicitly described peptides, which analogs and/or derivatives may have the same or at least 5 similar properties and or activity. On the other hand analogs which counteract the activity of the explicitly described peptides are also within the skill of the art, given the teaching of the present invention. Therefor derivatives and/or analogs, be it of the same or different length, be it agonist 10 or antagonist, be it peptide-like or peptidomimetic, are part of the scope of this invention.

A preferred embodiment of the present invention is the peptide with the sequence YIGEVLVSV which induces lysis of the cell presenting it at a very low concentration of peptide present. 15 This does not imply that peptides inducing lysis at higher concentrations are not suitable. This will for a large part depend on the application and on other properties of the peptides, which were not all testable within the scope of the present invention.

20 The peptides and other molecules according to the invention find their utility in that they may be used to induce tolerance of the donor immune system in HA-2 negative donors, so that residual peripheral blood lymphocytes in the eventually transplanted organ or the bone marrow, as it may be 25 does not respond to host HA-2 material in a HA-2 positive recipient. In this way GvHD may be prevented. On the other hand tolerance may be induced in HA-2 negative recipients in basically the same way, so that upon receipt of an organ or

bone marrow from an HA-2 positive donor no rejection on the basis of the HA-2 material occurs. It may be the case that the HA-2 peptide acts in a non-allelic restricted manner. In that case the tolerance induction is not restricted to HA-2
5 negative individuals.

For tolerance induction very small doses can be given repeatedly, for instance intravenously, but other routes of administration may very well be suitable too. Another possibility is the repeated oral administration of high doses
10 of the peptides. The peptides may be given alone, or in combination with other peptides, or as part of larger molecules, or coupled to carrier materials in any suitable excipients.

Further applications of the peptide or derivatives
15 thereof lie in the prophylactic administration of such to transplanted individuals to prevent GvHD. This can be done with either agonists, possibly in combination with an adjuvant, or with antagonists which may block the responsible cells. This can be done with or without the concomittant
20 administration of cytokines.

Furthermore the peptides according to the invention can be used to prepare therapeutic agents capable of eliminating a subset of cells, directly or indirectly, especially cells of hematopoietic origin. This can be illustrated by the following
25 examples, which refer to leukemia related therapeutic agents.

- a) A HA-2 positive recipient (in bone marrow transplantation) can be subjected to an additional pre bone marrow transplant conditioning regime. This means that an

agent which specifically recognizes a peptide according to the invention (a HA-2 peptide) as presented on hematopoietic cells, which agent induces elimination of the cells presenting said peptide, is administered to the recipient before 5 transplantation. This agent will eliminate all residual cells (leukemic cells) of hematopoietic origin. Such agent include but are not limited to T cells (preferably provided with a suicide gene) and/or antibodies coupled to toxic moieties.

b) A HA-2 negative donor for Bone marrow transplantation can 10 be vaccinated with a peptide according to the invention, a HA-2 peptide. Upon transplantation to a HA-2 positive recipient, the donor's immune system can eliminate any residual or recurrent HA-2 peptide presenting cells in the recipient which are of course leukemic.

c) A transplanted HA-2 positive recipient, transplanted with 15 HA-2 negative (or for that matter HA-2 positive) bone marrow and suffering from recurrent disease (relapse), i.e. HA-2 positive leukemic cells, can be treated with (again) an agent which specifically recognizes a peptide according to the invention (a HA-2 peptide) as presented on hematopoietic cells, which agent induces elimination of the cells presenting said peptide. In case of HA-2 positive bone marrow being 20 transplanted to the HA-2 positive recipient, it is still essential (in case of recurrent disease) to eliminate all HA-2 positive cells even though this includes the transplanted 25 material, because otherwise the HA-2 positive leukemia will kill the recipient. In the latter case the patient can be retransplanted, if necessary.

Diagnostic applications are clearly within the skill of the art. They include, but are not limited to HA-2 typing, detection of genetic aberrancies and the like.

Other therapeutical applications of the peptide include
5 the induction of tolerance to HA-2 proteins in HA-2 related (auto)immune diseases, such as possibly in Rheumatoid arthritis. On the other hand they may be used in vaccines in HA-2 related (auto)immune diseases.

On the basis of the peptide described herein genetic probes can be produced which can be used to screen for the gene encoding the protein. On the other hand such probes may be useful in detection kits as well. On the basis of the peptide described herein anti-idiotypic B cells and/or T cells and antibodies can be produced. All these embodiments have
10 been made possible by the present disclosure and therefor are part of the present invention.

The techniques to produce these embodiments are all within the skill of the art.

Dose ranges of peptides and antibodies and/or other molecules
20 according to the invention to be used in the therapeutical applications as described hereinbefore are usually designed on the basis of rising dose studies in the clinic. The doses for peptides may lie between about 0.1 and 1000 µg per kg bodyweight, preferably between about 1 and 10 µg per kg
25 bodyweight.

The invention will be described in more explanatory detail in the following experimental part.

EXPERIMENTAL

Using mHag specific CTL clones as in vitro tools, some murine and human mHag have been isolated from MHC molecules by 5 acid elution and were shown to be peptides presented by MHC molecules (13,14). Further characterization i.e. the exact amino acid sequence of the mHag peptides and the identification of the proteins from which these mHag originate, have sofar not been reported. Only a small number 10 of 'non-conventional defined' murine mHag, like the H-3 encoded β 2 microglobulin alleles (15) and the Hmt restricted mitochondrial encoded maternally transmitted antigen (16), have been characterized. Here we report the identification, by tandem mass spectrometry, of the HLA-A2.1 restricted mHag HA-2 15 epitope.

To isolate the mHag HA-2, HLA-A2.1 molecules were purified by affinity chromatography from HLA-A2.1 positive Epstein Barr Virus (EBV)-transformed B lymphocytes (EBV-BLCL) expressing HA-2. The HLA-A2.1 bound peptides were isolated by 20 acid treatment followed by 10kD filtration (14). These low molecular mass molecules were fractionated by reverse phase HPLC and individual fractions were analyzed for mHag HA-2 sensitizing activity by incubation with the mHag HA-2 negative, HLA-A2.1 positive lymphoblastoid cell-line T2 in a 25 ^{51}Cr release assay. One fraction (fraction 33) sensitized T2 for lysis by the HA-2 specific CTL clone 5H17 (17) (Figure 1a). When this fraction was rechromatographed using a shallower gradient, HA-2 sensitizing activity was observed in

fractions 37 and 38 (Figure 1b). However, as assessed using microcapillary HPLC/electrospray ionisation tandem mass spectrometry, the latter fractions still contained over 100 different HLA-A2 binding peptides (18). To determine which of 5 the peptides was responsible for the HA-2 sensitizing activity, fraction 37 was analyzed using an on-line splitter (19), allowing comparison of the mass spectrometric data with results of the functional assay. Figure 2a shows a single peak of HA-2 sensitizing activity in four adjacent wells. From the 10 many peptides present in these wells, the relative ion abundance profile of four peptides (with mass to charge ratios (m/z) of 651, 869, 979, 1000) matched the activity profile of the HA-2 specific CTL activity. Collision activated dissociation (CAD) analysis performed for the species with m/z 15 979 revealed the existence of 2 different peptides, YXGEVXVSV and SXDFGTXQV (figure 3a and 3b). The X stands for L or I, which cannot be distinguished by mass spectrometry under these conditions. Synthetic peptide mixtures were made with an equimolar mixture of L and I in place of X and assayed for 20 HA-2 specific sensitizing CTL activity. Only incubation with peptide mixture YXGEVXVSV resulted in lysis of T2 (20).

In order to further define the natural processed peptide, four single peptides were synthesized with I or L at positions two and six and microcapillary HPLC coelution studies of these 25 synthetic peptides and the isolated fraction were performed. Peptide YIGEVIVSV did not coelute with the natural processed peptide and can therefore be excluded as the natural processed epitope, whereas the other three peptides, YIGEVLVSV,

YLGEVLVSV and YLGEVIVSV did coelute (21). These three peptides all sensitized the T2 cell line for lysis by clone 5H17 (Figure 4a). Peptide YIGEVLVSV induced 50% lysis at a concentration of 40 pM, whereas these concentrations were 5 substantially higher for peptides YLGEVLVSV and YLGEVIVSV (1.5 nM and 2.25 nM). All three concentrations are within the range of 10 pM-50 nM established for other naturally processed epitopes (19,22). Clone 5H13 is an independently derived CTL that, based on panel analysis, also recognizes HA-2, but 10 differs slightly in its fine specificity of antigen recognition from 5H17 (10,23). Clone 5H13 also recognized all 3 peptide variants (Figure 4b). While the concentration of peptides necessary to give half-maximal epitope reconstitution were 5-10 fold higher than for 5H17, peptide YIGEVLVSV still 15 sensitized at 100 fold lower concentrations than the other two. These results establish that, despite their fine specificity differences (10,23), both HA-2 specific CTL recognize the same peptide epitope.

Binding studies with these three peptides showed that 20 peptide YIGEVLVSV was the highest binder to HLA-A2.1. The concentration that resulted in 50% inhibition of the binding of the iodinated standard peptide to purified HLA-A2.1 was 5.6 nM, while those for YLGEVIVSV and YLGEVLVSV were 9.5 and 15 nM respectively (Figure 5). These values place these 25 peptides among the highest affinity naturally processed peptides that have been defined so far (24). However, the differences in binding affinities for these three peptides is merely a factor of 3. The fact that YIGEVLVSV sensitizes for

recognition by clones 5H17 and 5H13 at 50-100 fold lower concentrations than the other two peptides indicates that this peptide is recognized with highest affinity by the TCR and thus may be the actual HA-2 epitope.

5 A search of DNA and protein sequence databases led to two human sequences that both matched at 7 out of 9 residues to peptide YIGEVLVSV. Peptide YYGEVGVSV is derived from oligodendrocyte myelin glycoprotein (25) and peptide YIGSVLISV was from unconventional myosin IC (26). Both human peptides
10 were synthesized and tested for sensitizing activity. Only the myosin IC derived peptide YIGSVLISV could sensitize T2 cells for lysis by 5H17 and 5H13 with a 50% lysis inducing concentration of 5-50 nM (27). Human unconventional myosin IC belongs to a large family of myosin genes (28,29), that
15 consist of different classes and that are indicated to be involved in cell locomotion and organelle transport (28,29). All cell types probably express several myosins from each class simultaneously. Tissue restricted distribution of some myosins has been reported (26,29). Database searches showed
20 that in different class I myosins of various origin, ranging from Acanthamoeba castellanii to human, this peptide sequence showed conservation for Y, I, G, V, and V at position 1, 2, 3, 5 and 9. Notably, the HA-2 peptide sequence differs in the nonconserved amino acid positions from the myosin IC peptide
25 sequence. Human unconventional myosin IC is the only cloned human class I myosin gene, but there is evidence for the presence of at least 2 other class I myosins in human cells. Therefore, it is not unlikely that an as yet unknown class I

myosin protein containing YIGEVLVSV is present in humans.

Interestingly, ongoing studies demonstrate the evolutionary conservation of several mHag, including HA-2, between human and non-human primates (30). Because mHag HA-2 is only 5 presented by haematopoietic cells, this unknown class I myosin is either restricted to haematopoietic cells or is only presented by haematopoietic cells because of tissue specific processing.

The polymorphism of mHag HA-2 is an intriguing issue. 95% 10 of the HLA-A2.1 positive population expresses the HA-2. Consequently, the HA-2 specific CTL were generated in vivo between a mHag HA-2 disparate bone marrow donor/recipient combination. The HA-2 polymorphism can be explained by either mutations in or adjacent to the HA-2 gene or by polymorphism 15 of the antigen processing system.

Until now, information on mHag has been extremely scarce. Although the physiological function of mHag is still unknown, their pivotal role in organ transplantation in general, and in bone marrow transplantation in particular, is undeniable. We 20 herewith report, to our knowledge for the first time, the amino acid sequence of a mHag defined by GvHD-derived CTL. The availability of the mHag peptide sequence may allow in vivo modification of the GvHD related T cell responses.

Furthermore, since mHag HA-2 is expressed on cells of the 25 hematopoietic lineage including leukemic cells, it is a candidate for immunotherapy of leukemia prior to bone marrow transplantation.

Fig. 5. Binding of synthetic peptides to purified HLA-A2.1. HPLC purified peptides were assayed for the ability to inhibit the binding of iodinated hepatitis B core antigen peptide, FLPSDYFPSV, to purified HLA-A2.1 molecules as previously described (23). (closed circles), YIGEVLVSV; (closed triangles), YLGEVLVSV; (closed squares), YLGEVIVSV; (closed diamonds), the influenza M1 protein antigen GILGFVFTL. All data points are the average of at least two independent experiments.

References

1. Goulmy E, Gratama JW, Blokland E, Zwaan FE, van Rood JJ
5 (1983) A Minor transplantation antigen detected by MHC restricted cytotoxic T lymphocytes during graft-versus-host-disease. *Nature* 302: 159-161.
2. Tsoi M-S, Storb R, Dobbs S, Medill I, Thomas ED (1980). Cell mediated immunity to non-HLA antigens of the host by
10 donor lymphocytes in patients with chronic graft-vs-host disease. *J. Immunol.* 125: 2258-2262.
3. Tsoi M-S, Storb R, Santos E, Thomas ED (1983) Anti-host cytotoxic cells in patients with acute graft-versus-host disease after HLA identical marrow grafting. *Transplant Proc.* 15: 1484-1486.
4. Irlé C, Beatty PG, Mickelson E, Thomas ED, Hansen JA
(1985) Alloreactive T cell responses between HLA identical siblings. *Transplantation* 40: 329-333.
5. Van Els C, Bakker A, Zwinderman AH, Zwaan FE, van Rood
20 JJ, Goulmy E (1990) Effector mechanisms in GvHD in response to minor Histocompatibility antigens. I. Absence of correlation with CTLs. *Transplantation* 50: 62-66.
6. Irscheck E, Hladik T, Niederwieser D et al. (1992) Studies
25 on the mechanism of tolerance or Graft-versus-Host Disease in allogeneic bone marrow recipients at the level of cytotoxic T cell precursor frequencies. *Blood* 79: 1622-1628.

7. Niederwieser D, Grassegger A, Auböck J, Herold M, Nachbaur D, Rosenmayr A, Gächter A, Nussbaumer W, Gaggl S, Ritter M and Huber C (1993) Correlation of minor histocompatibility antigen specific cytotoxic T lymphocytes with Graft-versus-Host Disease status and analyses of tissue distribution of their target antigens. Blood, 81: 2200-2208.
8. Goulmy E. Class-I restricted human cytotoxic T lymphocytes directed against transplantation antigens and their possible role in organ transplantation (1985). Prog. in allergy, vol. 36: 44-72.
9. Schreuder GMTH., Pool J, Blokland E, Van Els C, Bakker A, Van Rood JJ and Goulmy E. Genetic analysis of human minor Histocompatibility antigens demonstrates Mendelian segregation independent from HLA (1993). Immunogenetics 38: 98-105.
10. Van Els C, D'Amaro J, Pool J, Bakker A, van den Elsen PJ, Van Rood JJ and Goulmy E (1992) Immunogenetics of human minor Histocompatibility antigens: their polymorphism and immunodominance. Immunogenetics 35: 161-165.
11. De Bueger M, Bakker A, Van Rood JJ, Van der Woude F and Goulmy E. (1992). Tissue distribution of human minor Histocompatibility antigen. Ubiquitous versus restricted tissue distribution indicates heterogeneity among human CTLs defined non-MHC antigens. J. Immunology 1992, 149: 1788-1794.
12. Van Lochem EG, Van de Keur M, Mommaas M, de Gast G and Goulmy E. (1994). Expression of cytotoxic T cell defined

minor Histocompatibility antigens on human peripheral blood dendritic cells and skin derived Langerhans cells, manuscript submitted for publication.

13. Marijt WAF, Veenhof WFJ, Goulmy E, Willemze R, Van Rood JJ and Falkenburg JHF (1993). Minor histocompatibility antigen HA-1, -2, -4 and HY specific cytotoxic T cell clones inhibit human hematopoietic progenitor cell growth by a mechanism that is dependent on direct cell-cell contact. *Blood* 82: 3778-3785.

10 14. Falkenburg F, Goselink H, van der Harst D, Van Luxemburg-Heijs SAP, Kooy-Winkelhaar YMC, Faber LM, de Kroon J, Brand A, Fibbe WE, Willemze R and Goulmy E (1991). Growth inhibition of clonogenic leukemic precursor cells by minor histocompatibility antigen-specific cytotoxic T lymphocytes. *J. Exp. Med.* 174: 27-33.

15. Van der Harst D, Goulmy E, Falkenburg JHF et al (1994). Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by cytotoxic T-cell clones. *Blood* 83: 1060-1066.

20 16. Den Haan J, Pool J, Blokland E, Bontrop R and Goulmy E (1994). Minor Histocompatibility antigens are conversed between primates. Manuscript in preparation.

17. Goulmy E, Schipper R, Pool J (1994). Minor histocompatibility antigen mismatches influence the development of GvHD after HLA genotypically identical bone marrow transplantation. Manuscript subm. for publication.

18. Rötzschke O, Falk K, Wallny H-J, Faath S and Rammensee H-G (1990). *Science* 249: 283.
19. Falk K, Rötzschke O and Rammensee H-G (1990). *Nature* 348: 248.
- 5 20. De Bueger M, Verreck F, Blokland E, Drijfhout J-W, Amens R, Koning F and Goulmy E (1993). Isolation of a HLA-A2.1 extracted human minor histocompatibility peptide (1993). *Eur. J. Immunol.* 23: 614-618.
- 10 21. Den Haan JJM, Blokland E, Koning F, Drijfhout J-W and Goulmy E (1994). Structure analysis of human minor histocompatibility antigens HA-1 and HA-2. Abstract NWO retraite.
22. Powis SJ, Twonsend RM, Deverson EV et al. (1991) *Nature* 354: 528.
- 15 23. Momburg F, Ortiz-Navarrete V, Neefjes J, Goulmy E, v.d. Wal Y, Spits H, Powis SJ, Butcher GW, Howard JC, Walden P and Hä默ling GJ (1992). The proteasome subunits encoded by the major histocompatibility complex are not essential for antigen presentation. *Nature* 360: 174-177.
- 20 24. H.J. Wallny and H-G Rammensee, *Nature* 343, 275 (1990). O. Rötzschke, K. Falk, H.J. Wallny, S. Faath, H-G. Rammensee, *Science* 249, 283 (1990). M. Sekimata, P. Griem, K. Egawa, H-G. Rammensee, M. Takiguchi, *Int. Immunol.* 4, 301 (1992). L. Franksson, M. Petersson, R. Kiessling, K. Karre, *Eur.J. Immunol.*, 23, 2606 (1993);
- 25 25. M. De Bueger et al. *Eur.J.Immunol.* 23, 614 (1993);
26. M.E. Kurtz, R.J. Graff, A. Adelman, D. Martin-Morgan, R.E. Click, *J. Immunol.* 135, 2847 (1985). H-G Rammensee,

P.J. Robinson, A. Grisanti, M.J. Bevan, *Nature* **319**, 502 (1986). B. Perarnau et al., *Nature* **346**, 751 (1990);

27. B. Loveland, C-R. Wang, H. Yonekawa, E. Hermel, K. Fischer Lindahl, *Cell*, **60**, 971 (1990);

5 28. The HA-2 specific CTL clone 5H17 originate from a female patient who underwent bone marrow transplantation for severe aplastic anaemia. The pre-transplant conditioning regime consisted of total lymphoid irradiation and cyclophosphamide. The patient was grafted with non-T-cell-depleted bone marrow from her HLA identical father. The patient suffered from severe acute GvHD grade III followed by extensive chronic GvHD. The HA-2 specific CTL clone was generated from post BMT PBL according to the protocol described earlier. E. Goulmy, in *Transplant*, Rev.J.Morris and N.L. Tilney, Eds. (Saunders Company 2, 29, 1988);

10 29. Data not shown;

30. A.L. Cox et al., *Science* **264**, 716 (1994);

31. Peptide mixtures YSGEVXVSV and SXDFGTXQV were tested in several concentrations against clone 5H17 and clone 5H13.

20 In addition to T2, an HA-2 negative HLA-A2.1 positive EBV-BLCL was used to present the peptide mixture;

32. Data not shown;

33. K. Udate, T.J. Tsomides, H.N. Eisen, *cell* **69**, 989 (1992). R.A. Henderson et al., *Proc. Natl. Acad. Sci.*, **90**, 10275 (1993). O. Mandelboim et al., *Nature*, **369**, 67 (1994), A. Uenaka et al., *J. Exp. Med.*, **180**, 1599 (1994);

25 34. 5H13 and 5H17 demonstrated identical patterns when analyzed against 100 healthy unrelated HLA-A2.1 positive

individuals. A discriminatory reaction pattern between the clones was noted when a target cell was analyzed expressing a natural HLA-A2 variant molecule;

35. Y. Chen et al., J. Immunol., 152, 2874 (1994). J. Ruppert
5 et al., Cell, 74, 829 (1993);

36.

37. W.M. Bement, T. Hasson, J.A. Wirth, R.E. Cheney,
M.S. Mooseker, Proc. Natl. Acad. Sci. USA, 91, 6549
(1994);

10 38. Peptide YYGEVCVSV was tested in a concentration range of
50 nM to 0.5 pM against 5H17 as well as 5H13. No activity
was found;

39. M.A. Titus, Curr. Opin. Cell Biol., 5, 77 (1993). E.
Coudrier, A. Durrbach, D. Louvard, FEBS, 307, 87 (1992);

15 40. M. Mooseker, Curr. Biol., 3, 245 (1993);

41. J.M.M. den Haan, J. Pool, E. Blokland, R. Bontrop, E.
Goulmy, manuscript in preparation.

CLAIMS

1. A peptide constituting a T-cell epitope obtainable from the minor Histocompatibility antigen HA-2 comprising the sequence YXGEVXVSV or a derivative thereof having similar immunological properties, wherein X represents a leucine or an isoleucine residue.
5
2. An immunogenic polypeptide obtainable from the minor Histocompatibility antigen HA-2 comprising the sequence YXGEVXVSV or a derivative thereof, wherein X represents a leucine or an isoleucine residue.
- 10 3. A peptide or polypeptide according to claim 1 or 2, comprising the sequence YIGEVLVSV.
4. A peptide or polypeptide according to claim 1 or 2, comprising the sequence YLGEVLVSV or YLGEVIVSV.
- 15 5. Vaccine comprising an epitope or a polypeptide according to any one of claims 1-4.
5. A pharmaceutical formulation comprising an epitope or a polypeptide according to any one of claims 1-4.
6. Peptide or polypeptide according to claims 1-4 for use as a medicine.
- 20 7. Use of a peptide or polypeptide according to claims 1-4 in the preparation of a medicament for the induction of tolerance for transplants to prevent rejection and/or Graft versus Host disease.
8. A method for the elimination of a group of (neoplastic)
25 hematopoietic cells presenting a peptide in the context of HLA

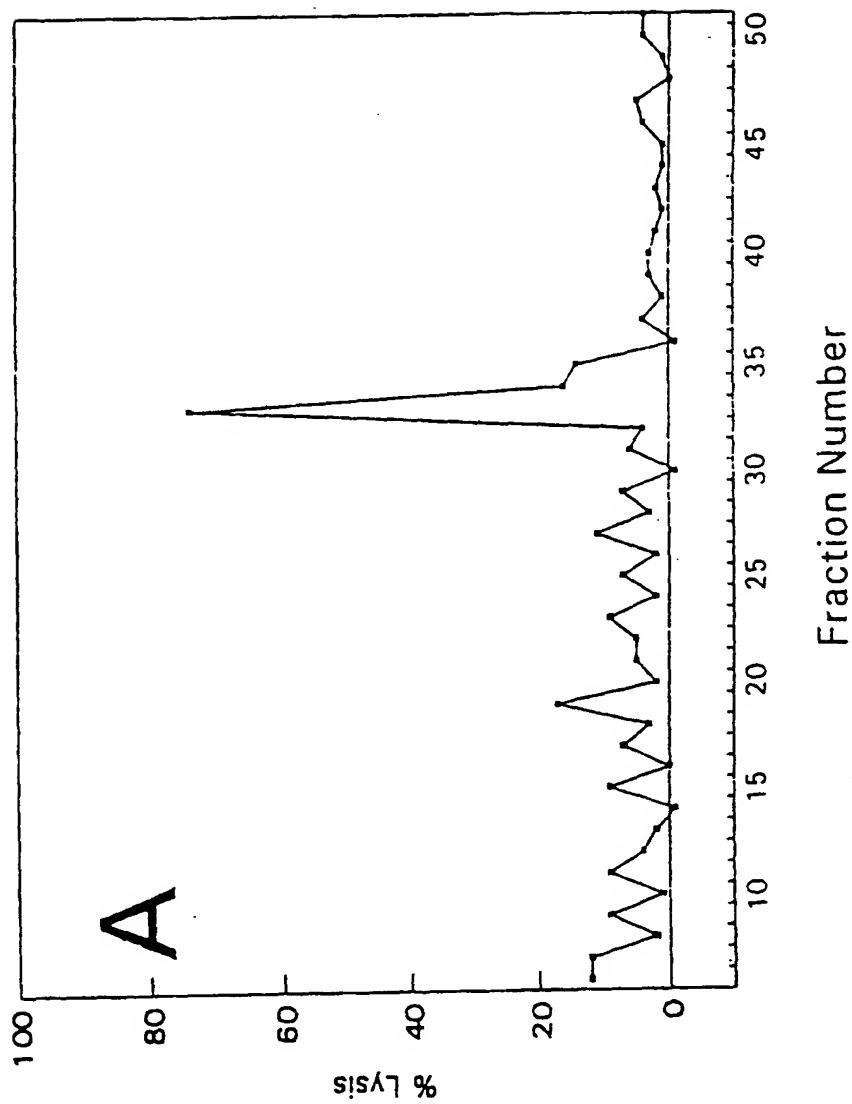
class 1 according to any of one of claims 1-4, whereby elimination is induced directly or indirectly by specific recognition of said peptide in said context.

9. Analog of the peptide according to claim 1, which is an antagonist for the activity of T cells recognizing said peptide.

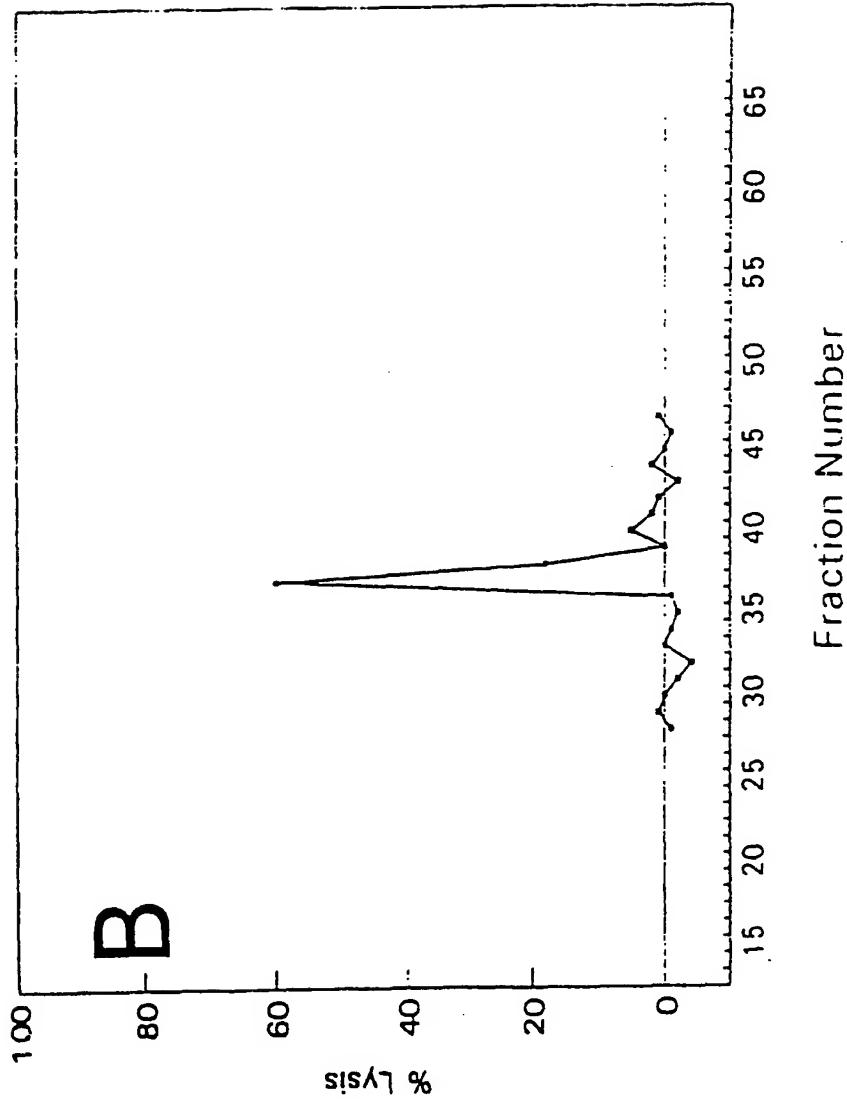
10. Method for the generation of antibodies, T cell receptors, anti-idiotypic B-cells or T-cells, comprising the step of immunization of a mammal with a peptide or a polypeptide according to claim 1 or 2.

11. Antibodies, T-cell receptors, B-cells or T-cells obtainable by the method of claim 9.

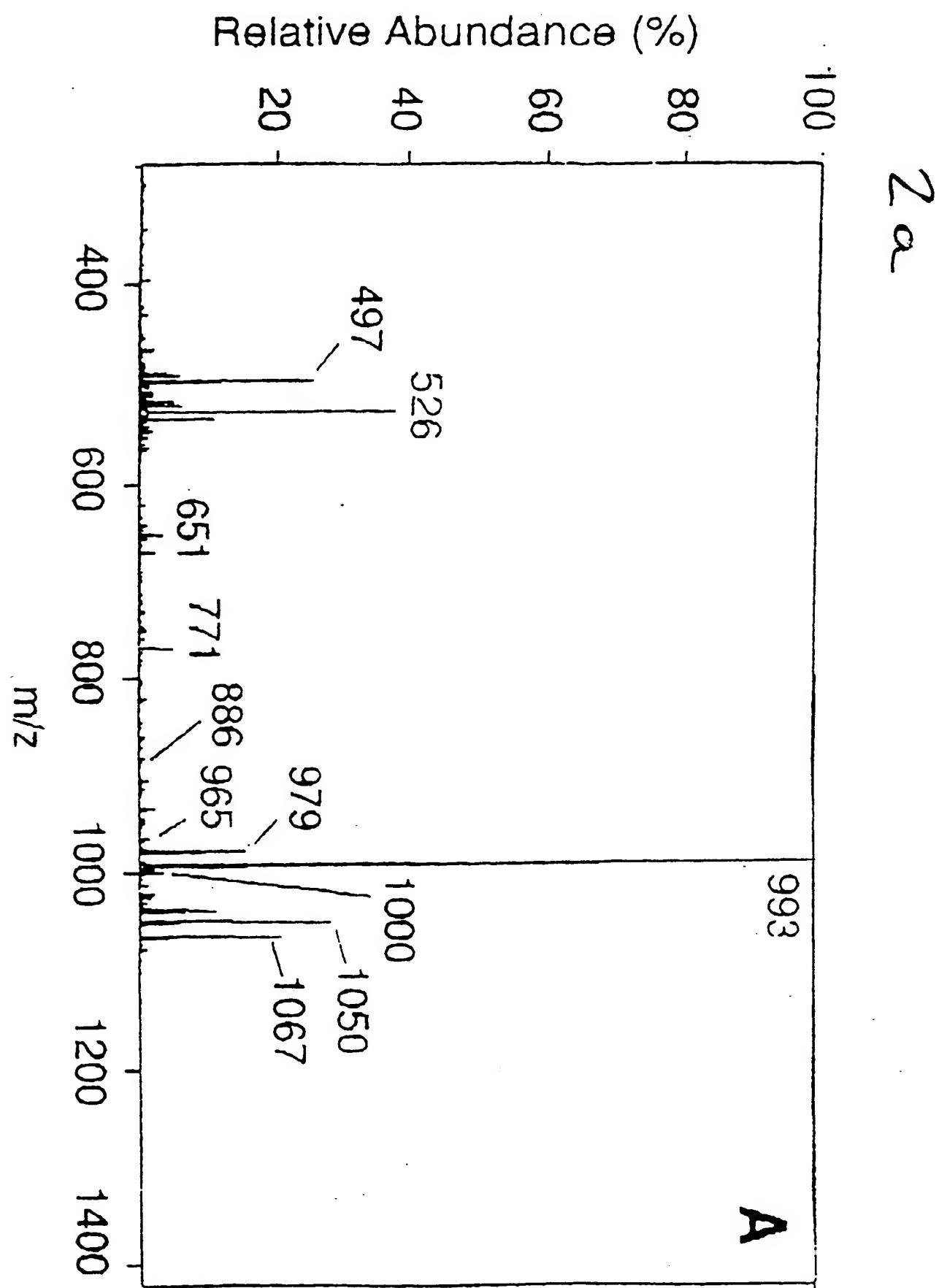
1A

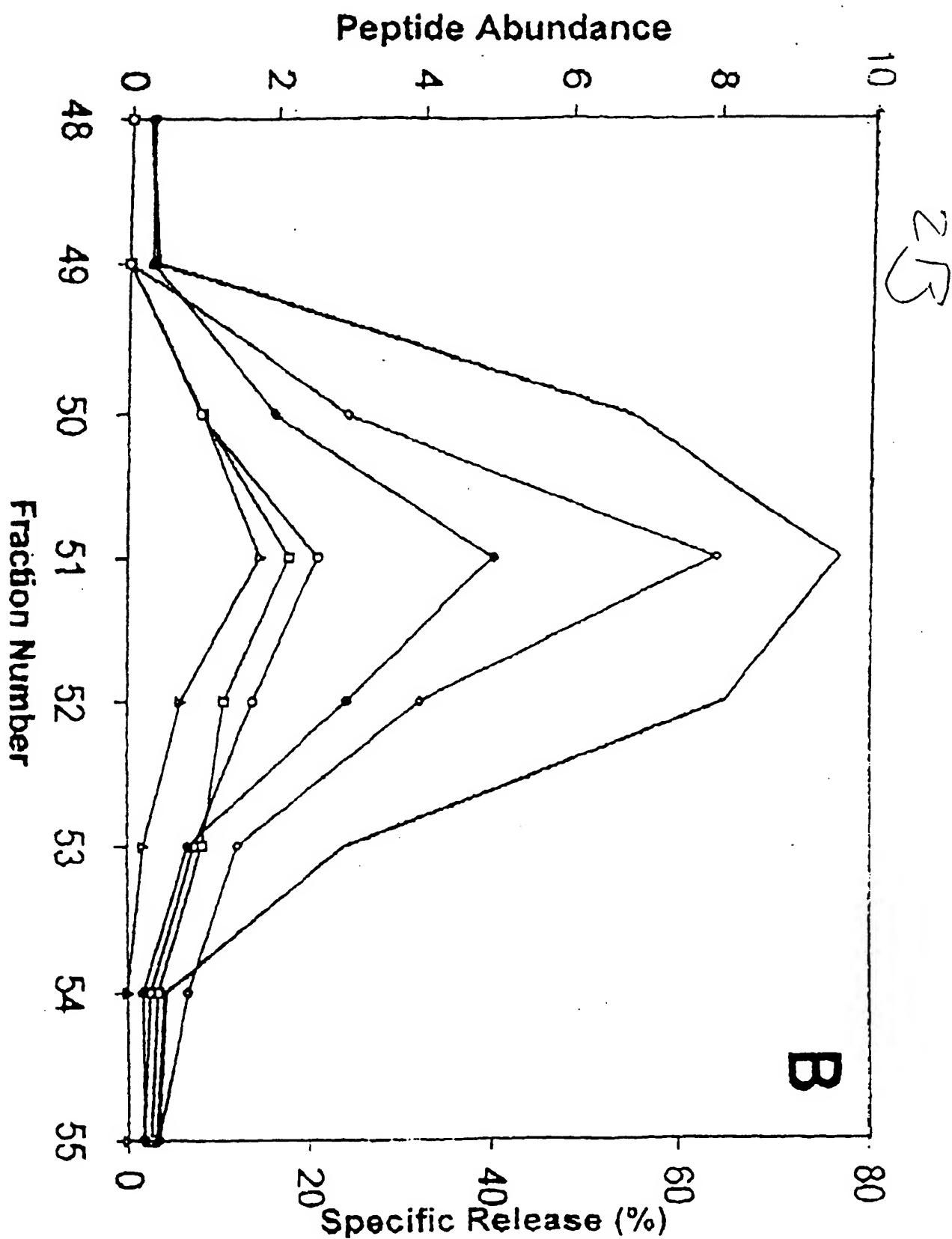


IB

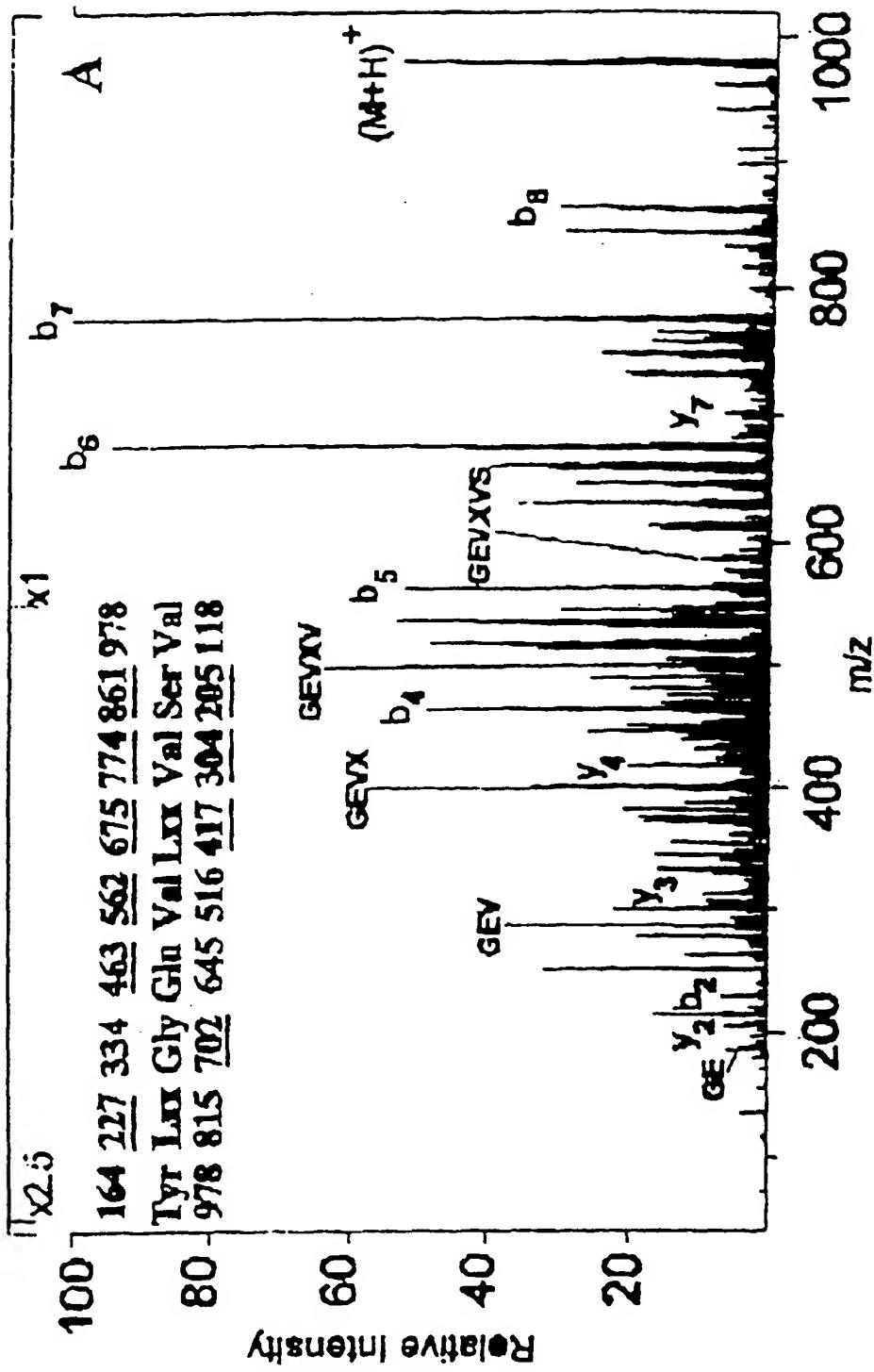


3/9



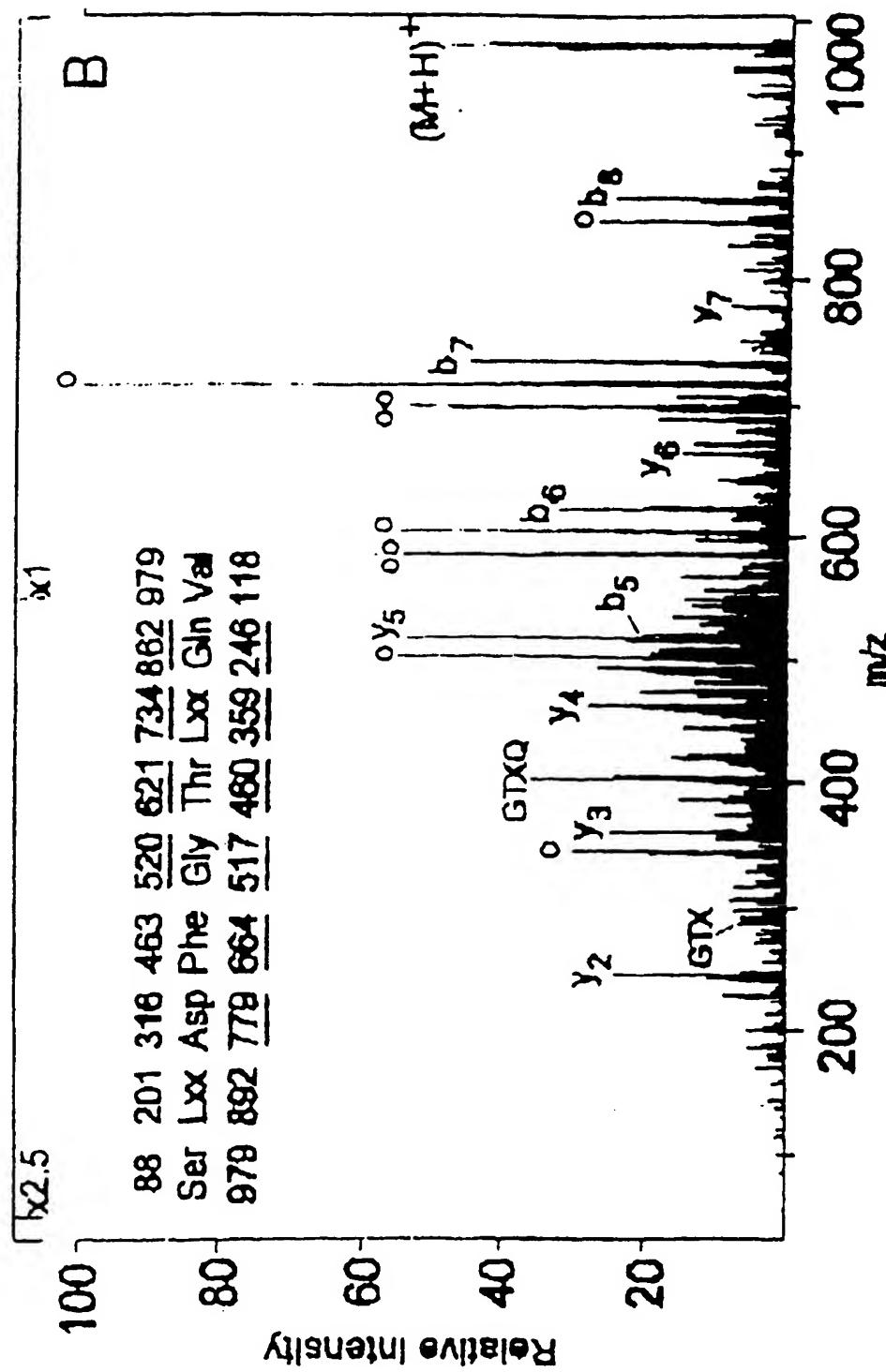


3 A

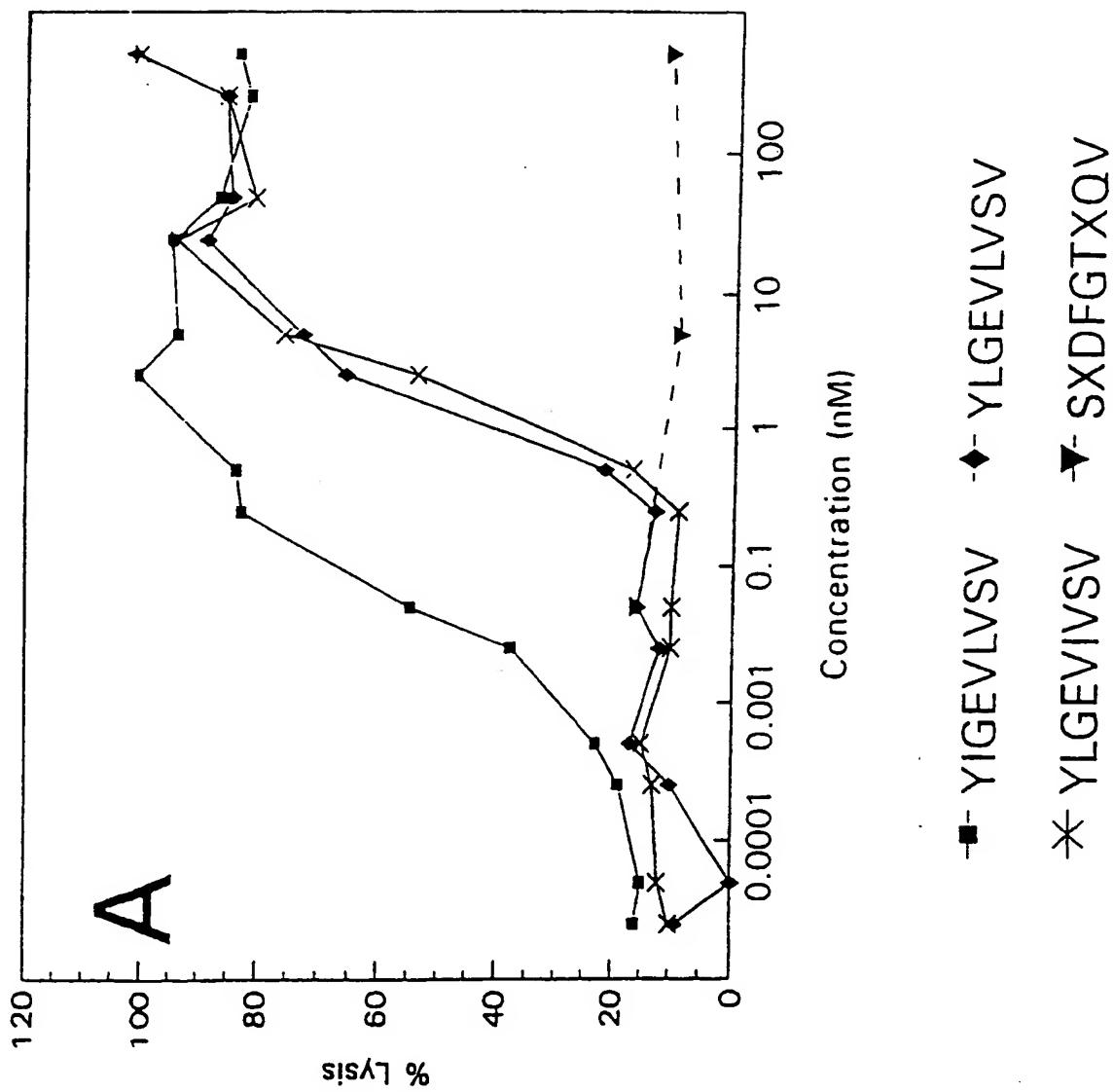


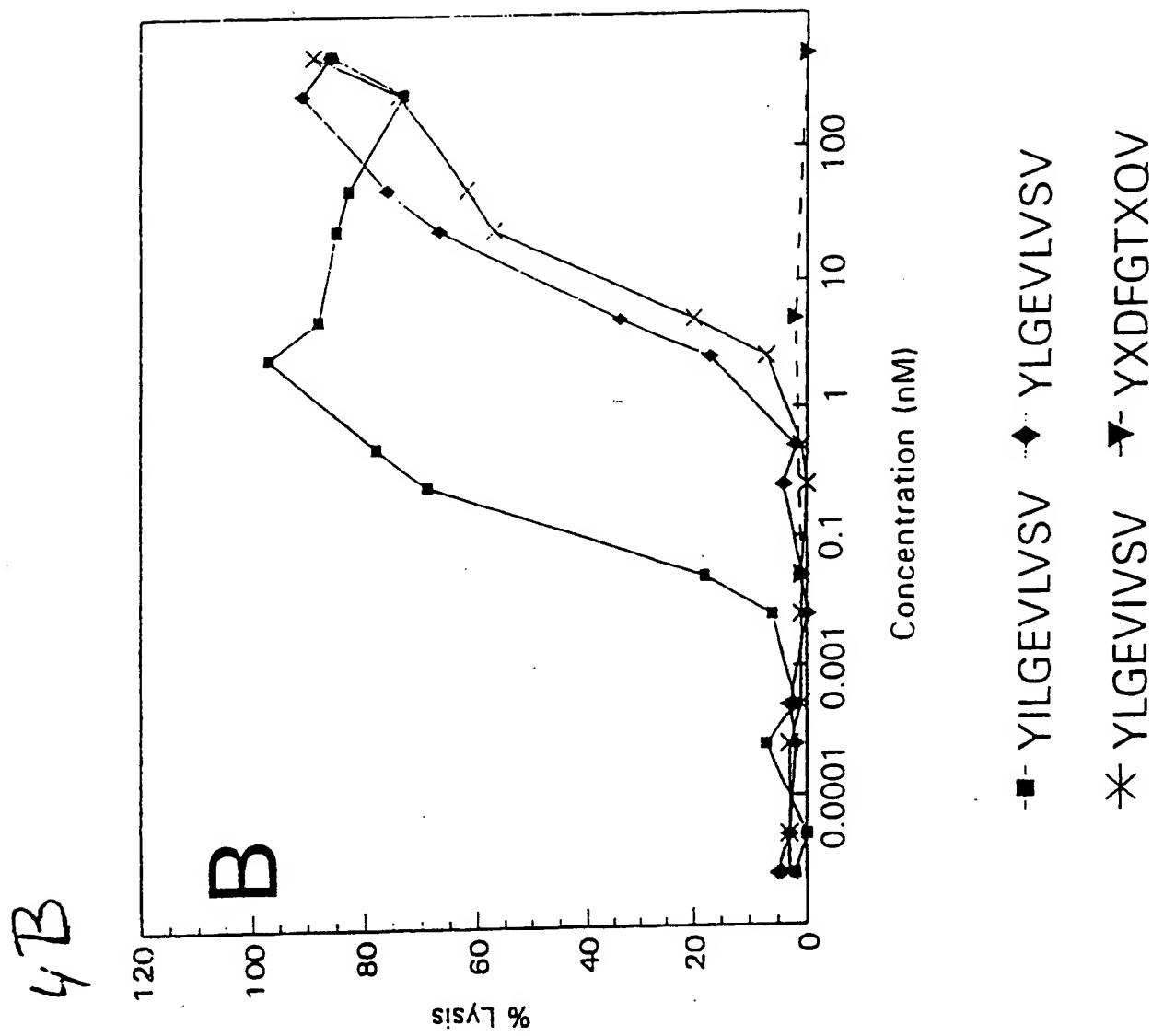
6/9

3 B



4A





9/9

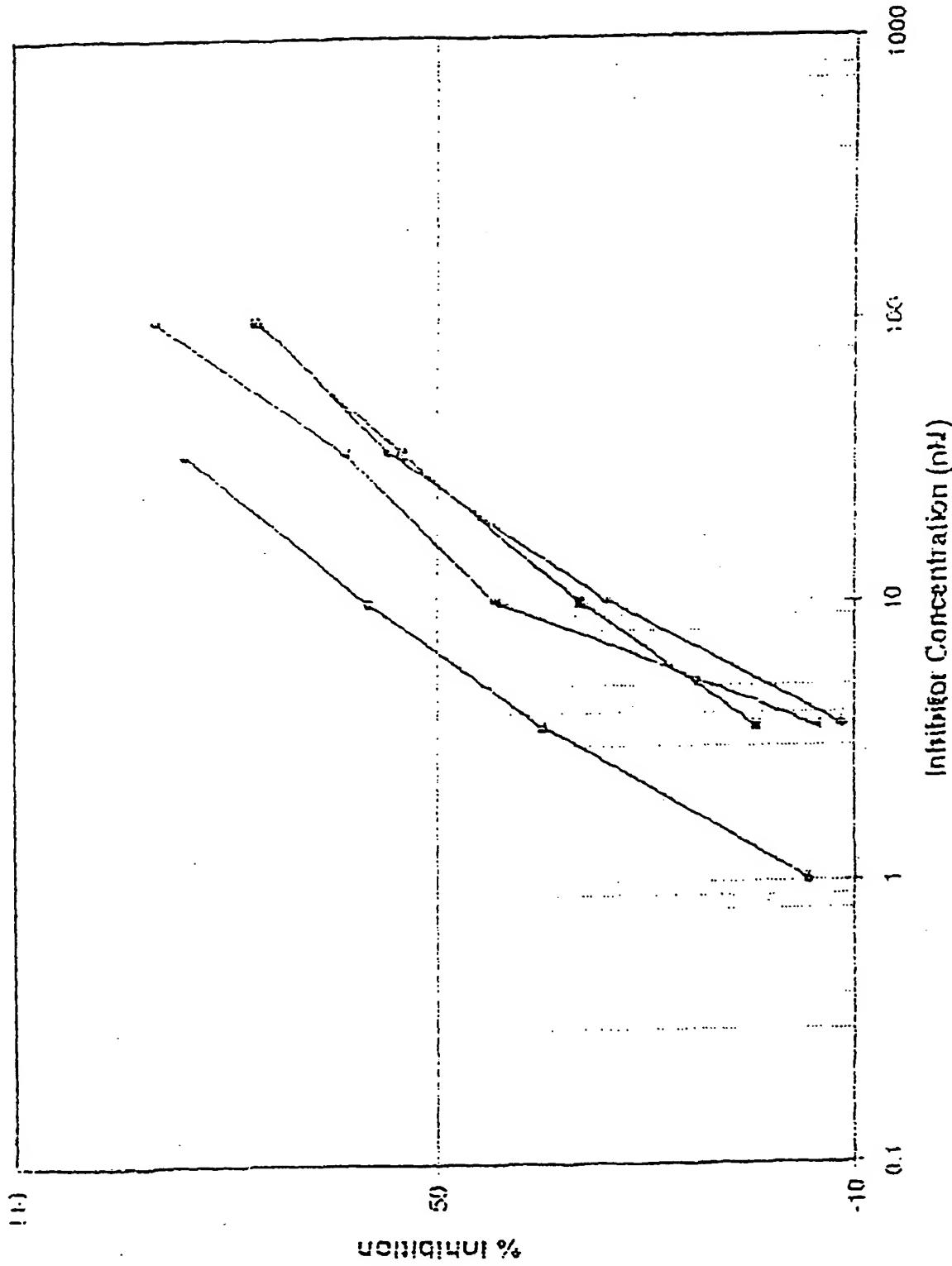


Fig 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 96/00183

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/74 C07K16/28 A61K38/16 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CELL, vol. 65, no. 4, 17 May 1991, NA US, pages 633-640, XP002013530 P GRIEM ET AL.: "Uneven tissue distribution of minor histocompatibility proteins versus peptides is caused by MHC expression" see the whole document --- -/-</p>	1-11

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

18 September 1996

Date of mailing of the international search report

24.09.96

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+ 31-70) 340-3016

Authorized officer

Masturzo, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 96/00183

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CHEMICAL ABSTRACTS, vol. 120, no. 1, 3 January 1994 Columbus, Ohio, US; abstract no. 6418k, LI YIN ET AL.: "Few peptides dominate cytotoxic T lymphocyte responses to single and multiple minor histocompatibility antigens" page 734; XP002013531 see abstract & INT. IMMUNOL., vol. 5, no. 9, 1993, pages 1003-1009,</p> <p style="text-align: center;">---</p>	1-11
A	<p>J CELL BIOL., vol. 129, no. 3, May 1995, NEW YORK, pages 819-830, XP002013599 H-E STÖFFLER ET AL.: "A novel mammalian myosin I from rat with an SH3 domain localizes to Con-A-inducible, F-actin-rich structures at cell-cell contacts" see the whole document</p> <p style="text-align: center;">-----</p>	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NL96/00183

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 8 because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although this claim (at least partially) refers to a method of treatment of the human body, the search was carried out and based on the alleged effects of the compounds.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

